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Effect of Bruising and Aging on the Alcohol-Insoluble Solids of Red Tart Cherries

Richard H. Golder, Sheila M. Levin, George R. Schacterle, and Robert L. Pollack*

Labeled acetate, citrate, and glucose were administered through the stems of carefully picked cherries. The cherries were then bruised and permitted to metabolize for 2 or 24 hr. The bruised cherries contained 1.55% alcohol-insoluble solids at 24 hr, and their unbruised controls contained 1.29%. The change in the alcohol-insoluble solids in the bruised from that of the control was due to increases in the cellulose and lignin fractions, not in the pectin. Glucose incorporation increased from 167 to 250% in all fractions in the controls between the 2- and 24-hr periods, but showed differences of only from 7 to 110% in the bruised cherries with low or no statistical significance. At 2 hr, acetate and citrate levels were above glucose levels, and were affected much less by bruising and aging.

The bruising and aging of Montmorency cherries have been shown to affect the firmness, water-holding capacity, and other characteristics in complex ways, especially in combination with other treatments (Whittenberger, 1952; Hills *et al.*, 1963; Buch *et al.*, 1961). Previous studies, which helped to elucidate some of the biochemical changes underlying these effects, showed that bruising altered respiration and conversion of carbon from acetate and citrate to carbon dioxide (Pollack *et al.*, 1958a,b, 1965) and induced callose formation (Dekazos and Worley, 1967). The studies presented here show further effects of bruising on the overall composition and substrate incorporation into the structural material of the cherries.

Temple University School of Pharmacy, Philadelphia, Pennsylvania 19140.

EXPERIMENTAL

On three alternate days during the harvest season cherries were harvested, as described previously by Pollack *et al.* (1958b), by cutting the stems gently while supporting the cherries carefully with a cotton pad. The harvested cherries were treated in groups of ten, and the cherries in each group were subsequently combined for homogenization and extraction.

To administer the substrates, 10-μl amounts of the substrates were placed in micro test tubes (about 3 mm $\times$ 10 mm). The stems of the cherries were then inserted into the solutions

Table I. The Effect of Bruising and Aging on Content of Alcohol-Insoluble Solids and Its Components in Red Tart Cherries

Incubation time	Percentage (g of each component per 100 g wet cherry wt)								Percent change 24 hr *vs.* 2 hr			
	2 hr				24 hr							
	Normal		Bruised		Normal		Bruised		Normal		Bruised	
AIS[a]	1.22 ±	0.09[b]	1.33 ±	0.07	1.29 ±	0.04	1.55 ±	0.09	6	<0.05[c]	17	<0.001
Pectin	0.59	0.08	0.53	0.03	0.64	0.05	0.52	0.03	8	<0.2	−2	<0.5
Lignin	0.34	0.07	0.41	0.05	0.32	0.07	0.54	0.04	−6	<0.8	32	<0.001
Cellulose	0.29	0.03	0.38	0.03	0.34	0.03	0.49	0.05	17	<0.02	29	<0.001

[a] Alcohol-insoluble solids. [b] Standard deviation. [c] Probability of difference being due to statistical variability according to standard "t" test.

Table II. Incorporation of the Different Substrates into the Cherry Fractions

Incubation time	Percent of applied radioactivity recovered in the fraction								Percent change 24 hr *vs.* 2 hr			
	2 hr				24 hr							
	Normal		Bruised		Normal		Bruised		Normal		Bruised	
Glucose-U-^{14}C												
AIS[a]	0.47 ±	0.09[b]	0.35 ±	0.10	1.29 ±	0.35	0.53 ±	0.11	174	<0.05[c]	51	...
Pectin	0.18	0.02	0.14	0.04	0.48	0.12	0.18	0.13	167	<0.02	29	...
Lignin	0.17	0.04	0.12	0.04	0.48	0.03	0.21	0.04	182	<0.001	75	...
Cellulose	0.06	0.01	0.06	0.02	0.21	0.07	0.13	0.05	250	<0.05	117	...
Citrate-1,5-^{14}C												
AIS	0.74 ±	0.07	0.78 ±	0.20	0.96 ±	0.06	0.79 ±	0.24	30	<0.001	1	...
Pectin	0.19	0.03	0.18	0.03	0.24	0.04	0.19	0.04	26	...	6	...
Lignin	0.54	0.04	0.48	0.17	0.58	0.18	0.52	0.10	7	...	8	...
Cellulose	0.09	0.03	0.15	0.04	0.19	0.07	0.22	0.07	110	<0.02	47	...
Acetate-1-^{14}C												
AIS	0.98 ±	0.46	1.04 ±	0.25	1.20 ±	0.18	1.32 ±	0.24	22	...	27	...
Pectin	0.35	0.11	0.38	0.03	0.42	0.04	0.44	0.68	20	...	16	...
Lignin	0.45	0.07	0.38	0.11	0.46	0.13	0.52	0.12	2	...	37	...
Cellulose	0.14	0.05	0.16	0.02	0.19	0.07	0.25	0.01	36	...	56	<0.001

[a] Alcohol-insoluble solids. [b] Standard deviation. [c] Probability of difference being due to statistical variability according to standard "t" test. [d] Probability less than 90% confidence level.

and left until the solution was drawn up into the stem and cherries. Two successive 10-μl portions of water were then added to each test tube to be drawn up and thus wash the labeled solution completely into the cherries.

With each day's harvest, separate sets of normal and bruised cherries, containing the labeled substrates, were simultaneously incubated. Analyses of each final batch of cherries were carried out in duplicate. The 2-hr interval was chosen to obtain early indications of any effects, and the 24-hr time period allowed effects to develop without allowing extensive deterioration to occur.

Some cherries were then bruised by rolling them between two glass plates until they became flaccid, taking care to avoid breaking the skin. The bruised fruits and their unbruised controls were left to stand at 30 ± 2°C for 2 or 24 hr. Following incubation the stems and seeds were removed and the cherries extracted with hot 80% (v/v) ethanol, including the cherry weights as part of the water, according to the procedure of Jermyn (1955). The alcohol-insoluble solids were fractionated by removing pectins with hot water, removing lignin with sodium chlorite and acetic acid, and leaving final cellulose residue (Jermyn, 1955).

The radioactive compounds administered were sodium acetate-1-^{14}C, 3.5 × 10^6 decompositions per minute (DPM)/cherry; citric acid-1,5-^{14}C, 2.4 × 10^6 DPM per cherry; and glucose-U-^{14}C, 110 × 10^6 DPM per cherry. They were purchased from Tracerlab, Inc. Samples were counted in a Packard Tri-Carb scintillation counter. Samples of the total alcohol-insoluble solids and of the cellulose were ground, suspended in a scintillation medium containing Cab-O-Sil, sonicated for 2 min, and then counted.

RESULTS AND DISCUSSION

The increase in content of total alcohol-insoluble solids in the bruised cherries, small at 2 hr and greater at 24 hr, is shown in Table I, and can be seen to be due to increases in the lignin and cellulose components only.

As can be seen in Table II, glucose is the only substrate used here whose uptake into all fractions tested was significantly affected by bruising and aging. Its uptake increased greatly on 24-hr incubation in the controls, but the bruised cherries showed much less and a less statistically significant increase. The lower 2-hr uptake of glucose than of acetate and citrate is contrary to what would be expected from the apparent need to convert acetate and citrate to glucose phosphate before their incorporation into the polysaccharides. No explanation for this phenomenon is presently available.

These studies thus confirm and extend earlier conclusions (Buch *et al.*, 1961) that bruising cherries does induce changes in their polysaccharide content. The differences shown here in the direction of change of pectin from that of lignin and cellulose may help explain some of the variability in the effect of bruising on water-holding capacity of the cherries. Callose (Dekazos and Worley, 1967) was not specifically sought in this study. It would be expected to comprise part of the cellulose fraction in the fractionation procedure and may contribute to the observed increase in cellulose content.

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